CHLORINE
CHEMISTRY
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Dr. C.W. Jameson
National Toxicology Program
Report on Carcinogens
MD WC-05, P.O. Box 12233
Research Triangle Park, NC 27709

VIA FACSIMILE AND FEDERAL EXPRESS

Dear Dr. Jameson:

A
Council
of the
Chemical
Manufacturers
Association

The Chlorine Chemistry Council (CCC), a business council of the Chemical Manufacturers Association, is pleased to submit the enclosed comments in response to NTP's evaluation of dioxin (2,3,7,8-TCDD) for the 9th Report on Carcinogens. CCC is dedicated to addressing public policy issues related to chlorine chemistry, and therefore has a significant interest in NTP's evaluation of TCDD.

As discussed more fully in the enclosed comments, the currently available epidemiology and mechanistic data are not sufficient for NTP to reclassify TCDD as a known human carcinogen. The epidemiological weight-of-evidence for TCDD does not adequately fulfill the well-recognized causation criteria. In fact, EPA, SAB, and IARC have independently concluded that the available epidemiological data on TCDD carcinogenicity are "limited" and do not support classification of TCDD as a known human carcinogen. NTP's revised listing criteria allow for consideration of mechanistic data. However, beyond Ah receptor binding and enzyme induction, the mechanism by which TCDD induces tumors in animals is not well understood. Even less is known about TCDD's mechanism of action in humans. Therefore, the available mechanistic data does not support classifying TCDD as a "known" human carcinogen.

Thank you for your consideration of these comments. If you have any questions regarding this submission, please contact Mr. David Fischer at (703) 741-5179.

Sincerely,

Clifford "Kip" Howlett, Jr.
CCC Executive Director
CMA Vice President

1300 Wilson Blvd.
Arlington, VA, USA
22209
Tel: 703-741-5000
Fax: 703-741-6095

Comments of the Chlorine Chemistry Council on the National Toxicology Program's Classification of 2,3,7,8-TCDD in the Ninth Report on Carcinogens

Introduction

The National Toxicology Program's (NTP's) current *Biennial Report on Carcinogens* (Report) lists 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as *Reasonably Anticipated to be a Humans Carcinogen.* For the ninth edition of this report, NTP is proposing to change the listing of TCDD to the *Known to Be a Human Carcinogen* category. In response to NTP's request for public comment, (62 Fed. Reg. 37,272 (July 11, 1997)), the Chlorine Chemistry Council (CCC), a business council of the Chemical Manufacturers Association, submits the following comments concerning the proposed TCDD listing.

NTP's recently revised criteria for listing chemicals as known human carcinogens (61 Fed. Reg. 50,499 (September 26, 1996)) requires "sufficient evidence of carcinogenicity from studies in humans which indicate a causal relationship between exposure to the agent . . . and human cancer." As discussed below, the epidemiology data for TCDD do not establish a causal relationship between TCDD exposure and cancer. The studies reporting an association between TCDD exposure and human cancers suffer from confounding, inconsistency, lack of specificity, and weak associations. Indeed, the U.S. Environmental Protection Agency (EPA), EPA's Science Advisory Board (SAB) and an International Agency for Research on Cancer (IARC) Epidemiology Working Group have concluded that the epidemiological data for TCDD are only "limited" or "suggestive." Therefore, TCDD epidemiology data do not support a change in NTP's current carcinogen listing for TCDD.

The recently revised NTP criteria also state that data related to a substance's mechanism of action may be considered. The criteria do not state whether mechanistic data could be used in place of epidemiological data that are not "sufficient" and, if so, how such data should be weighed. Before NTP can rely on mechanistic data for an animal carcinogen to assume carcinogenicity in humans, NTP must demonstrate that the substance exerts its carcinogenic effect through the same mechanism in both animals and humans. In the case of TCDD, sufficient mechanistic data do not exist.

CCC urges NTP not to change its current carcinogen listing for TCDD. NTP, however, should update its TCDD exposure and production discussion in its ninth edition of the Report. As discussed below, new TCDD production and exposure information demonstrates a dramatic decrease in TCDD production and exposure over the past decade, and TCDD production and emissions are anticipated to decrease further as new and proposed regulatory requirements are implemented.

The Epidemiological Data on TCDD-Induced Cancer in Human Populations Are Not Sufficient for NTP to Reclassify TCDD as a Known Human Carcinogen

The numerous epidemiological studies of human populations (even those exposed to unusually high levels of TCDD) do not demonstrate that TCDD is a known human carcinogen. Indeed, EPA, after a lengthy and careful analysis, decided not to classify TCDD as a Group A carcinogen. The SAB supported this decision, stating "...virtually all of the Committee believes that the studies of humans [would be categorized] as "limited" providing for an overall evaluation...as "Probably Carcinogenic to Humans with limited supporting information from human studies" (*An SAB Report: A Second Look at Dioxin*, p. 67). In addition, based on a thorough review of the epidemiology data, including a number of studies not available to EPA or the SAB during the preparation and review of the Draft *Dioxin Reassessment*, the IARC Epidemiology Working Group recently concluded that the epidemiological data were "limited" and therefore, by themselves, did not support a recommendation to designate TCDD a known human carcinogen. (IARC relied on mechanistic data to support its reclassification of TCDD as a "known" human carcinogen. Significant weaknesses with this mechanistic data are discussed below.)

The chemicals currently classified by NTP as known human carcinogens all have been placed in that category based on epidemiology data which satisfies the rigorous causation criteria established and recognized by the scientific community. There is no ongoing debate concerning whether any of these chemicals belongs on the list. The data are clear and unambiguous. The same standards should apply to TCDD or any other chemical which NTP considers classifying as a known human carcinogen.

NTP's category of *Known To Be A Human Carcinogen* is based primarily on sufficient evidence of carcinogenicity from studies in humans which indicates a "causal relationship" between the agent and cancer. Causal relationships, by definition, imply that the data for any chemicals which are in this category meet the formal causation criteria, including strength of association, dose-response relationship, biological plausibility, consistency and specificity. Compounds currently classified by NTP as *Known To Be A Human Carcinogen* substantially fulfill the causation criteria. This group includes arsenic, asbestos, benzene, benzidine, bis(chloromethyl) ether, chloromethyl methyl ether, chromium (VI), coke oven emissions, diethylstilbestrol, lead arsenate, nickel, nickel sulfide roasting and dust, and vinyl chloride, Indeed, these substances are classified as known human carcinogens because the epidemiological data are of sufficient quality and quantity to fulfill these criteria. Table 1 illustrates the essential basis for including several representative chemicals in the known human carcinogen category.

Unlike NTP's current list of *Known Human Carcinogens*, the weight-of-the-evidence for TCDD does not adequately fulfill the well recognized causation criteria. Indeed, EPA, SAB and IARC have concluded that the totality of the epidemiological data on TCDD carcinogenicity are only "limited" or "suggestive." Clearly, the data for the chemicals presently classified by NTP as *Known Human Carcinogens* rise above the level of being merely "limited" or "suggestive."

Table 1

Summary of Causation Criteria Fulfillment For Representative Chemicals Currently Classified by NTP as Known Human Carcinogens and TCDD

Compound	Consistent Findings	Strong Association	Dose-Response	Decreased Risk with Decreased Exposure
Arsenic and lead arsenate	+	+	+	+
Asbestos	+	+	+	-
Benzene	+	+	+	-
Benzidine	+	+	-	+
BCME & CMME	+	+	+	-
Chromium (VI)	+	+	+	-
Coke oven emissions	+	+	+	+ (?)
DES	+	+	-	-
Nickel, roasting and dust	+	+	+	+
Vinyl chloride	+	+	+	+
TCDD	-	-	-	-

Epidemiological Studies Reviewed in the Draft Dioxin Reassessment

EPA cites four key epidemiological studies in it's Draft *Dioxin Reassessment* as the best evidence demonstrating the carcinogenicity of TCDD (Fingerhut et al. 1991, Saracci et al. 1991, Zober et al. 1990, and Manz et al. 1991). All involve workers occupationally exposed, many of whom had chloracne (indicating very high exposures). The Fingerhut study reported an excess of respiratory cancers and all cancers combined. The authors concluded that the elevated risk for "all cancers combined" was consistent with the carcinogenic effects of TCDD observed in animal

studies. However, not a single tumor observed in the animal studies was found (i.e., cancers of the liver, biliary passages, gall bladder or nasal passages). Nor were cancers predicted from previous epidemiological studies (cancer of the stomach, Hodgkin's Disease, and non-Hodgkin's lymphoma) observed. Likewise, the studies by Saracci et al., Zober et al., and Manz et al. essentially failed to report "cancers of a priori interest." In reviewing these data, EPA concluded that "[t]he epidemiology evidence for a TCDD lung cancer hazard in humans is suggestive, but not conclusive, while that for all cancers combined has less certainty" (Draft Chapter 8, *Dose-Response Modeling,* p. 121).

The NIOSH Study By Fingergut et al.

Because of its size and detailed exposure data, the NIOSH study by Fingerhut et al. (1991) is generally held to be the best study of an occupational cohort highly exposed to TCDD. However, despite its size and detail, it is questionable whether the NIOSH study, because of the well recognized problems of confounding and bias, supports an association between TCDD exposure and cancer. Indeed, the SAB was skeptical about the results of this "best" study noting that workers "were exposed to a wide variety of potentially carcinogenic agents in addition to dioxin." The SAB stated that "[g]iven the possible confounding, and the somewhat equivocal links of dioxin to excess cancer in the group as a whole, it is difficult to document a dioxin-cancer relationship" (SAB, p. 51). Even the NIOSH study itself acknowledged that its findings could not "exclude a possible contribution from factors such as other occupational chemicals or smoking," that "other chemical exposures to which the workers were exposed may confound this analysis," and that "it is difficult to separate the effects of exposure to TCDD-contaminated products from the effects of exposure to numerous other chemicals encountered while employed at the plants" (Fingerhut et al. 1991, pp. 3, 18, and 28).

Exposures to other substances in the workplace could have easily accounted for any observed cancer increases in the NIOSH cohort. For example, smoking and exposure to asbestos could have accounted for the observed increase in lung cancers. Indeed, a misclassification of a single case of mesothelioma as a respiratory cancer would likely eliminate the statistical significance of the lung cancer finding attributed to TCDD exposure in the NIOSH high exposure cohort. Mesothelioma is typically under diagnosed in populations with no obvious exposure to

asbestos, so it is not unreasonable to hypothesize that one more case of mesothelioma might have escaped notice in the high exposure NIOSH cohort (Selikoff and Seidman 1992). This raises important questions concerning interactive effects between smoking and exposure to asbestos that could have substantially affected the interpretation that the moderately increased risk of lung cancer was due to TCDD exposure.

Other elements of the NIOSH study also suggest confounding by other chemicals. For example, the NIOSH study combined the results from 12 different manufacturing facilities in which workers could have been exposed to TCDD. However, all cancers and lung cancers were significantly elevated only at one plant (plant #10). The mortality experiences from Plant #10 contributed significantly to the overall pattern observed in the combined cohort. Removing the data from Plant #10 from the cohort brings the standard mortality ratio (SMR) for all cancers in the total cohort below statistical significance. Removal of data from Plant #10 also decreases the SMRs for all cancers and lung cancer in the high exposure cohort. Workers at this plant had a median 1.5 years in TCDD-contaminated processes and 21 additional years employed at the same plant with potential exposure to at least 13 additional chemicals classified by various agencies as potential human carcinogens. It is necessary to consider the contribution of other potentially carcinogenic chemicals to the incidence of cancer at plant #10. The NIOSH study, however, failed to do so.

Collectively, all of the studies of highly exposed populations reviewed by EPA in the Draft *Dioxin Reassessment* are more or less deficient in fulfilling three of the primary causation criteria (strength of association, consistent findings, and dose-response). For example, contrary to expectation, these studies of highly exposed occupational cohorts do not demonstrate effects more conclusively than studies of less exposed cohorts. Further, even among these studies, there is a lack of consistency in the incidence of statistically significant cancer endpoints. This is illustrated in Table 2.

Table 2

Lack of Consistency in Reported Associations Between Exposure to TCDD, and Cancer at Various Sites

Study	Cancer Type Reported				
	All Cancers	Lung	STS	Thyroid	Stomach
Fingerhut	+[6]	+[1,6]	+[5]	-	-
Saracci	-	-	-	+	+
Zober	-[3], +[4]	-	-	-	-
Manz	+[2]	+[1,2]	-	--	-

1 Confounded by smoking and exposure to asbestos
2 Not significant with both comparison control groups
3 Three cohorts based on job descriptions
4 Single cohort based on presence of chloracne or erythema
5 Cases only seen in 2 of 12 plants studied; significance questioned by authors
6 Only significant in 1 of 12 plants studied; 13 other potential carcinogens known to be present at same plant

Recent Epidemiology Studies Reviewed by IARC

A number of studies have been published since the release of the Draft *Dioxin Reassessment* and SAB's critical review of that document. The IARC Epidemiology Working Group considered many of these studies. In addition to the NIOSH study from the United States (Fingerhut et al. 1991), IARC also considered studies in the Netherlands (Bueno de Mesquita et al. 1993) and Germany (Ott and Zober 1996 and Becher et al. 1996), and an international study by IARC in which these and other data were aggregated (Kogevinas et al. 1996). In addition, IARC considered the most recent study of the Seveso cohort (Bertazzi et al. 1996). The IARC Epidemiology Working Group found that even these additional studies were not sufficient to alter the conclusion that the epidemiological data were still "limited" and failed to demonstrate a causal relationship between exposure to TCDD and human cancer. Each of these studies are briefly described below.

- Bueno de Mesquita et al. (1993) studied a cohort from the Netherlands. The cohort, part of the *IARC International Register of Persons Exposed to Phenoxy Herbicides and Contaminants,* was composed of 2310 workers engaged in the manufacture of phenoxy herbicides, chlorophenols and related compounds, many of which were contaminated with TCDD. Exposure estimates were based on detailed occupational history rather than on measurements of serum TCDD levels. No statistically significant increase in mortality due to all cancers combined or to respiratory cancer was observed. Significantly, the authors concluded that their findings "suggest that the increases in cancer mortality among workers exposed to phenoxy herbicides and chlorophenols may be attributable to chance" (Bueno de Mesquita et al. 1993, p. 289).

- Becher et al. (1996) studied four occupational cohorts from Germany consisting of a total of 2479 workers from four plants employed in manufacturing chlorophenoxy herbicides or chlorophenols. Exposure of the entire cohort was based on measured TCDD levels in a subsample of workers from two of the plants. The authors observed an increased mortality from all cancers combined in one of the four cohorts (SMR 134; CI 109-164). In addition, they reported an increased mortality from cancer of the buccal cavity and pharynx in one cohort (SMR 822; CI 300-1789), although this cohort was not the same one in which excess respiratory cancer was observed. Similarly, excess mortality from non-Hodgkin's lymphoma (NHL) was observed in two of four cohorts. Lung cancer (SMR 102; CI 102-213) or respiratory cancer (SMR 115; CI 115-230) was only elevated in one of the four cohorts. While smoking status was known for one cohort, this was not the cohort in which the elevated mortality from lung cancer was observed. Because these findings involve exposures to chemicals other than TCDD, it is difficult to attribute the observed effects to TCDD. For example, in discussing a slight excess of bladder cancer in one cohort, the authors noted that this could have been due to exposure to aromatic amines such as o-toluidine, ethyl toluidine, aniline, and anisidine which were known to have been used in that plant. Also, these findings are at odds with the reported findings of the NIOSH study in which no excess NHL or cancer of the buccal cavity and pharynx were reported, suggesting that different substances may have induced the cancers observed in the two cohorts.

- Ott and Zober (1996) reported cancer mortality in a small (N=243) occupationally exposed German cohort. Exposure was based on chloracne status and measured TCDD serum levels in a subsample of the cohort. Smoking status (never smoked, former smoker, current smoker) was based on a survey of an unknown number of cohort members. Although the authors reported 11 total cases of lung cancer, only one reported never having smoked cigarettes. The significance of the lung cancer findings is unclear since there was no TCDD dose-response trend among non-smokers. With respect to cancer mortality, the authors reported no statistically significant increases in any cancer associated with increasing exposure to TCDD. Finally, for cancer incidence, there were no statistically significant increased standardized incidence ratios (SIRs) for any cancer site associated with increasing TCDD dose levels. The authors of this study concluded, "[u]nfortunately, with such a small cohort, the risk estimates are not very stable and we are unable to assess whether TCDD might be exerting an influence independent of other

cancer risk factors." Thus, the results of this study provide little, if any, evidence that TCDD might be a human carcinogen. In addition, the authors' failure to find an increased incidence of all cancers combined or of respiratory cancer contradict the results of other studies.

- Bertazzi et al. (1996) provided an update of cancer mortality in the Seveso population exposed to TCDD after 15 years of latency. Exposure estimates were based on proximity to the accident with highest exposures in Zone A, intermediate exposures in Zone B and no exposure in Zone R. The authors reported no significant increase in respiratory cancer or in all cancer combined in men or women in any exposure zone. For women, they reported significant increased mortality from Hodgkin's disease and myeloma in Zone B, while for men they reported significant increased mortality from rectal cancer and leukemia in Zone B and increased mortality for esophageal cancer in Zone R. No increased cancer mortalities were observed in the highest exposed group. The authors of this study (which has not been peer reviewed) concluded only that "the observed departures from expectation, although based on small numbers of deaths, might be associated with dioxin exposure" (Bertazzi et al. 1996, p. 297).

- Kogevinas et al. (1996) reported cancer mortality in the IARC international retrospective cohort study of manufacturing workers or sprayers exposed to phenoxy herbicides. Reconstructed exposures were based on job descriptions or on TCDD serum measurements in some cohorts. For workers exposed to any phenoxy herbicide or chlorphenol, the authors reported a marginally significant increase in all cancers in men (SMR 1.07; CI 1.01-1.13), but not in women. In workers exposed to phenoxy herbicides contaminated with TCDD, the authors reported marginally significant increases in all cancer combined (SMR 1.6; CI 1.05-2.35) and breast cancer (SMR 2.22; CI 1.11-3.98). Due to the well recognized problems of confounding with exposures to other chemicals, the marginally significant results in this study (which has not been peer reviewed) are difficult to attribute to TCDD exposure.

The Ranch Hand Study

The Ranch Hand Study (Wolff et al. 1995), which IARC's Epidemiology Working Group did not consider, provides findings in a cohort moderately exposed to TCDD. The large cohort which comprises this study, while not as heavily exposed to TCDD as the occupational cohorts noted above, provides valuable information suggesting that cancer might not be an endpoint of concern for typical (even elevated) exposures to TCDD. In 1992, 20 years after the last exposure to TCDD, the Ranch Hand cohort demonstrated no statistically significant group differences for any neoplasm. Wolff et al. (1995) concluded that "at the end of a decade of surveillance and more than 20 years after the last exposure to Agent Orange in Vietnam, Ranch Hands and Comparisons appear to be at equal risk for the development of all forms of neoplastic

disease and there ~~is~~ no evidence to suggest a positive dose-response relationship between body burden of dioxin and neoplastic disease."

Conclusion

The body of epidemiology evidence for TCDD, including recent studies examined by IARC, do not provide sufficient evidence that TCDD causes cancer in humans. Some human epidemiology studies, such as the Ranch Hand study (Wolff et al., 1995), suggest that low to moderate TCDD exposure may not be carcinogenic to humans. The evidence concerning the potential carcinogenic effects associated with high human TCDD exposures, on the other hand, is not clear. Studies of highly exposed industrial cohorts typically suffer from confounding by exposures to a wide variety of industrial chemicals (many of which are known to be potential carcinogens), inconsistency, and lack of specificity. The finding of "new" cancers in different cohorts, the failure to confirm key findings from previous studies (e.g., lung cancer), and the finding of only marginally elevated SMRs in cohorts exposed to TCDD levels do not provide a sufficient basis from which to conclude that TCDD is a known human carcinogen.

Current Information Concerning the Mechanism of Action for TCDD-Induced Carcinogenicity Does Not Support Changing the TCDD Carcinogenicity Listing

NTP's recently revised listing criteria state that mechanism of action may also be considered when drawing conclusions regarding carcinogenicity in humans or experimental animals. It is not clear whether mechanistic data could be used when epidemiological data are insufficient to move a chemical from the *Reasonably Anticipated to be a Human Carcinogen* to the *Known Human Carcinogen* list. Indeed, the only example provided in the revised criteria concerns removing an animal carcinogen from listing when evidence demonstrates that the substance, for mechanistic reasons, is not anticipated to be carcinogenic in humans.[1]

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[1] To date, very few chemicals have "cleared the hurdle" with respect to being classified (or not classified) as anticipated tobe human carcinogens or known human carcinogens based on mechanism of action data. Perhaps the best example of how data on mechanism of action have been used to **not** classify certain chemicals as potential human carcinogens concerns the issue of $\alpha_{2\mu}$-globulin nephropathy. For a certain class of chemicals (e.g., unleaded gasoline), chronic exposure results in nephropathy and renal tumors in male rats and no effects in female rats or in either sex of any other species. The nephropathy and tumor formation results from the

Presumably, if mechanistic data were used to move a substance from the *Reasonably Anticipated to be a Human Carcinogen* to the *Known Human* Carcinogen list, NTP must demonstrate that the mechanism in animals is at least qualitatively similar in both animals and humans. This would require a detailed understanding of the mechanism of action for a specific substance in both the experimental animals and humans. If NTP relied instead on inference and assumptions, it would likely find that most of the *Anticipated to be Human Carcinogens* would be upgraded to *Known Human Carcinogens*, thereby losing the important (and statutorily required) distinction between the two categories.

In the case of TCDD, little is known about its mechanism of action in animals and even less is known about its mechanism of action in humans. Therefore, current mechanistic data cannot support moving TCDD to the *Known Human Carcinogen* list. As discussed below, the current hypotheses concerning possible TCDD tumor-inducing mechanisms are merely speculation. What is known and generally accepted at this time concerning TCDD mechanisms of actions is that TCDD, like a number of other substances, most of which are not "known to be carcinogenic to humans," binds to the aryl hydrocarbon (Ah) receptor and results in the induction of certain enzymes. Neither of these events, however, are sufficient to explain how adverse responses, particularly cancer, occur. Apart from receptor binding and enzyme induction, little, if anything, is known, much less understood, about mechanistic events leading to TCDD-induced

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accumulation of $\alpha_{2\mu}$-globulin, a protein specific to male rats only. Since humans of both sexes lack this protein, there is no possibility of $\alpha_{2\mu}$-globulin-mediated renal nephropathy occurring in humans. Based on this well documented and unequivocal understanding of the mechanism of action, EPA's regulatory policy for chemicals that only induce renal tumors in male rats by this mechanism is that they do not pose a carcinogenic risk to humans. NTP's revised descriptive criteria for determining the potential carcinogenicity of chemicals implicitly endorses this body of data ("*...there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore reasonably be anticipated not to cause cancer in humans.*) This detailed and unequivocal understanding of the mechanism of action by which certain chemicals induce tumors in animals (and the validation of this in numerous peer reviewed publications) is in contrast to the current understanding of the mechanism of action by which TCDD might cause tumors in animals, much less in humans.

carcinogenicity in animals. Even less is known about the mechanisms by which TCDD might induce toxicity in humans.

The Draft Dioxin Reassessment Demonstrates That Little is Known About the Mechanism of Action by which TCDD Causes Cancer

EPA, after extensively reassessing TCDD, has acknowledged that little is known about the mechanism by which TCDD exerts toxicity (including cancer) in animals. For example, in it's Draft *Dioxin Reassessment* EPA states:

> [i]t has repeatedly been reported as the current opinion that all known effects of TCDD are probably Ah receptor mediated. However, except for the chain of events leading to the induction of certain enzymes, clear evidence for such a conclusion is still lacking" (p. 3-36).

A true mechanism of action for a chemical must be both necessary and sufficient with respect to explaining how the chemical induces toxicity in animals. For the two events of Ah receptor binding and enzyme induction for TCDD, this is clearly not the case. EPA has stated that "[b]inding to the Ah receptor appears to be necessary for all well-studied effects of dioxin but is not sufficient, in and of itself, to elicit these responses" (Draft *Dioxin Reassessment,* p. 9-78). It is clear from this statement that the mechanism of action by which TCDD induces toxicity is unknown. If binding to the Ah receptor is not sufficient, then some other explanation must address the critical data gap between Ah receptor binding and toxicity induction. Presently, there is little if any knowledge of events past Ah receptor binding which are involved with TCDD-induced carcinogenicity.

A number of statements by EPA (in it's Draft *Dioxin Reassessment* and in recent revisions of that document) and by the SAB (in reviewing the Draft *Dioxin Reassessment*) support the conclusion that Ah receptor binding and enzyme induction do not, in and of themselves, explain how adverse responses, particularly cancer, occur in animals. These statements also demonstrate that little is known about the mechanistic events leading to TCDD-induced carcinogenicity in animals and even less is known about the mechanism by which TCDD might induce toxicity in humans.

EPA states in the Draft *Dioxin Reassessment*:

In certain cases, no response occurs even when there is some receptor occupancy, a

> threshold phenomenon that reflects the biological "inertia" of the system (Ariens et al. 1960). In other cases a maximal response occurs well before all receptors are occupied, a phenomenon which reflects receptor "reserve" (Stephenson, 1956). Therefore, one cannot simply assume that the relationship between fractional receptor occupancy and biological response is linear. Furthermore, for a ligand (such as TCDD) that elicits multiple receptor-mediated effects, one cannot assume that the binding response relationship for one, simple effect (such as enzyme induction) will necessarily be identical to the binding response relationship for a different effect (such as cancer). (Chap. 2, at 2-5).

This statement undermines any conclusion that Ah receptor binding somehow "explains" the carcinogenicity of TCDD. If the binding response relationships for enzyme induction and cancer are different, it cannot be concluded with any confidence that the mechanism of action by which TCDD-induced carcinogenicity occurs is adequately understood.

Even assuming that the mechanism of carcinogenic action of TCDD in animals involves the Ah receptor, the lack of knowledge concerning the similarities and differences between human and animal Ah receptors undermines any speculation that the mechanism for the carcinogenicity of TCDD in animals applies to humans. According to EPA, there is great uncertainty on this critical point. EPA states that "[t]he human receptor has not been studied extensively, and it is unknown if the properties of the human protein differ substantially from those of the Ah receptor in animals (Draft *Dioxin Reassessment*, p. 2-8).

Further, EPA has concluded that "there is no clear mechanistic link between CYP1A1 induction and cancer" (Draft *Dioxin Reassessment,* p. 6-27). Because many chemicals, in addition to TCDD, can induce enzymes (e.g., CYP1A1), the role of this event in explaining carcinogenicity is unknown (particularly for non-genotoxic chemicals like TCDD). The fact that there is no clear link between Ah receptor-mediated CYP1A1 induction and cancer suggests that the mechanism by which TCDD induces cancer in animals is unknown.

The above examples highlight the fundamental uncertainty expressed in the Draft *Dioxin Reassessment* concerning the lack of understanding concerning the mechanism of TCDD-induced carcinogenicity. It is clear that beyond Ah receptor binding, the mechanism of action for TCDD-induced tumors in animals is poorly understood.

SAB's Review of the Draft Dioxin Reassessment Demonstrates Little is Known About the Mechanism of Action by Which TCDD Causes Cancer

The SAB's review of the Draft *Dioxin Reassessment* also supports the conclusion that the mechanism of TCDD-induced carcinogenicity is unknown or, at best, is poorly understood. The following excerpts from the SAB review clearly illustrate this point:

- "Chapter 2 [of the Draft *Dioxin Reassessment*] offers an unequivocal assessment that the Ah receptor mediates the biological effects of TCDD. Yet, when reading Chapters 3, 4 and 5 dealing with specific toxic events, it becomes clear that numerous fundamental uncertainties occur and mechanisms of action for the toxic events beyond receptor binding are largely unknown" (p. 49).

- "Much of what is purported to link the Ah receptor to specific toxic events is merely the demonstration of an association between the binding of TCDD to the receptor and an eventual appearance of an adverse effect some time later in some species.... But the possible downstream events, if they exist, between Ah receptor binding and the final toxic manifestation are not well established. Mechanism of action should mean that at least some of the intermediate steps, after Ah receptor binding and leading to the pathologic processes involved, are known to some extent.... In actual fact, the only mechanism of action involving the Ah receptor that has been worked out sufficiently well to be called the biological sequence that describes a "mechanism of action" is the induction of cytochrome P450.....The rest of the biological consequences of TCDD exposure are yet to be described adequately and sequentially in mechanistic terms" (p. 49).

- "[T]here is a large intellectual chasm between the elegant science describing the details of the TCDD receptor and its mechanism of initiating a cellular response, and the poorly understood manifestations of the toxic events associated with an alteration of the homeostasis of an animal. The linkage between Ah receptor action and specific cellular toxicity remains undefined.... In any future revisions, EPA should present more clearly the major deficiencies that exist in the current mechanism database and provide some discussion of any plausible alternative hypotheses" (p. 50).

- "Most of the "mechanistic data" support the involvement of the Ah receptor, but say little (in the context of toxicity), about how the activation of this protein alters normal physiologic function and/or development. Risk assessments based solely on Ah receptor activation or on the existing knowledge of CYP1A1 induction are unlikely to provide a biologically defensible prediction (quantitatively or qualitatively) of likely toxic outcomes in humans, particularly under low exposure scenarios" (p. 49).

- Concerning EPA's conclusion about the role that the purported mechanisms of action might contribute to the diversity of biological response seen in animals and, to some extent, in humans "can be better posed as...how convincing is the evidence for the purported mechanisms that link receptor binding to toxic effects in humans? Unfortunately, the

evidence is quite mixed" (p. 51).

- "The statement [EPA's conclusion that there is a continuum of responses, based, in part on speculations about mechanism of action] is only defensible in reference to a limited number of specific case examples, but cannot be taken as universally proven. Until a full mechanism of action has been mapped out, the reassessment's position remains unproved in general. The statement should not be presented as a "postulate (which is widely accepted as a universal truth not requiring proof) but as a current hypothesis (subject to change as new data are discovered)" (p. 79).

From the numerous above statements taken from the SAB's review of the Draft *Dioxin Reassessment* there can be no doubt that the mechanism of action by which TCDD induces tumors in animals is neither known nor understood. Given this uncertainty, it would be premature for NTP to proceed with the proposed reclassification of TCDD to a known human carcinogen based on mechanism of action considerations.

EPA's Recent Revisions to the Draft Dioxin Reassessment

EPA's recent revised Chapter 8 of the *Dioxin Reassessment* (Dose-Response Modeling, January 27, 1997) and it's partially revised risk characterization for the *Dioxin Reassessment* (Draft *Integrated Risk Characterization for TCDD,* Sept. 24, 1996) continue to demonstrate that the mechanism of action by which TCDD might induce tumors in laboratory animals is still unknown.

While Chapter 8 recognizes that TCDD binds to the Ah receptor, EPA expresses considerable uncertainty concerning this event. For example, EPA acknowledges that "[t]he relationship between Ah receptor binding and carcinogenicity of TCDD is less clear" (Revised Chap. 8, p. 6). EPA also states that, "[t]he induction of CYP1A proteins are perhaps the best characterized responses to dioxins. The relevance of these proteins to the toxic effects of TCDD are controversial" (Revised Chap. 8, p. 91). EPA also states:

- "Most of the mechanistic or dose-response information on dioxin's effects has been generated on changes in gene expression of single genes such as CYP1A1 induction. There is only limited information on the complete interaction of biochemical, molecular, and biological events that are necessary to produce a frank toxic effect such as cancer..." (p. 129).

- "The development and implementation of a complete mechanistic [risk assessment] model for the effects of TCDD identified several areas where future research is needed. Of critical utility would be data and models which are able to directly link gene expression with toxicity

in a mechanistic fashion" (p. 141).

In it's Draft *Integrated Risk Characterization for TCDD,* EPA further illustrates how poorly the mechanism of action by which TCDD might induce tumors in laboratory animals is understood. EPA states:

- "The fact that TCDD **may induce** a cascade of biochemical changes in the intact animal **raises the possibility** that dioxin **might produce** a response such as cancer by mechanisms that differ among tissues....One **possible mechanism** discussed is that TCDD **might activate** a gene(s) that is directly involved in tissue proliferation. A second mechanism involves TCDD-induced changes in hormone metabolism, which **may lead** to tissue proliferation ...which **might lead** to indirect mutagenic effects. Thus, while this reassessment as identified a number of **hypothetical mechanisms for cancer induction by TCDD, there remains considerable uncertainty about which mechanisms occur, with what levels of sensitivity, and in which species**" (p. 72, emphasis added).

- "The ability of TCDD...to modulate a number of biochemical parameters in a species-, tissue-, and temporal specific manner is well recognized. Despite the ever expanding list of these responses over the past 20 years and the elegant work on the molecular mechanisms mediating some of these, there still exists a considerable gap between our knowledge of these changes and the degree to which they are related to the biological and toxic endpoints elicited by these chemicals" (pp. 74-5).

- "Thus, while this reassessment has identified a number of hypothetical mechanisms for cancer induction by TCDD, there remains considerable uncertainty about which mechanisms occur, with what levels of sensitivity and in which species" (p. 72).

- "Thus, the mechanisms by which many, if not most, of the biochemical processes are altered by TCDD treatment remain to be determined. Nevertheless, it is presumed based on the cumulative evidence available...that all of these processes are mediated by the binding of TCDD to the Ah receptor" (p. 75).

Based upon these statements, it is simply not possible to support an argument that the mechanism by which TCDD induces cancer in laboratory animals (much less in humans) is known or understood.

Speculation Concerning Possible Mechanisms

The lack of knowledge concerning the mechanism of action by which TCDD induces tumors in animals, however, has not prevented EPA and others from speculating on a possible

mechanism. For example, in the Draft *Dioxin Reassessment,* EPA speculates that the carcinogenic actions of TCDD may involve hormones. EPA states that "the carcinogenic actions of TCDD involve a complex interaction of hormonal factors" (p. 9-43). However, a description of the sequence of hormonal events occurring after Ah receptor binding which lead to the carcinogenicity of TCDD in animals has never been published. Nor has the possible relevance of these events in humans. Even if hormone interactions play some role in high dose animal carcinogenicity, the role of hormone interactions, in potential human carcinogenicity is, at best, speculative. (Indeed, there is evidence that certain hormonal effects of TCDD act as an anti-carcinogen in humans at low doses.) The unknown nature of this aspect of TCDD carcinogenesis only adds to the uncertainty concerning the potential mechanism of action for TCDD.

EPA also speculates in it's Draft *Dioxin Reassessment* that there may exist some interplay between enzyme induction, hormonal effects and subsequent carcinogenicity. EPA states that:

> ...**it has been hypothesized** that increases in UDP-glucuronyltransferases leads to elimination of thyroxine and **may lead indirectly** to increased TSH synthesis...and hypertrophic responses by the thyroid. **There is speculation** that such prolonged stimulation **may lead** to the thyroid tumors seen in both rats and mice exposed to TCDD. **Data to confirm this effect of dioxin...in humans are not available** (p. 9-52, emphasis added).

The above speculation concerning a possible mechanism of action is not borne out by data. Thyroid cancer has almost never been reported in the TCDD-exposed cohorts studied. For example, in the NIOSH study (Fingerhut et al. 1989) there were no thyroid cancer deaths. Thus, empirical data demonstrates that this speculative mechanism of action for TCDD-induced tumors may be incorrect.

NTP Should Publish A Detailed Basis For Any Decision In Which Mechanism Of Action Considerations For TCDD-Induced Carcinogenesis Are Employed

Although IARC has classified TCDD as a known human carcinogen based on mechanistic considerations, a detailed explanation for this decision has yet to be published and subject to review by the scientific community. Therefore, it is not clear how IARC's decision can comport with the lack of scientific evidence. A decision by NTP to rely on mechanism of action data to raise TCDD to the category of *Known Human Carcinogen* must be based on a scientific

explanation acceptable to the scientific community. Given the considerable uncertainties (and controversy) surrounding this issue, and the general lack of any peer reviewed and published papers on the subject, it is incumbent upon NTP to publish a detailed basis for any decision in which mechanism of action considerations for TCDD-induced carcinogenesis are employed.

Conclusion

Based upon numerous statements from the Draft *Dioxin Reassessment*, the SAB's comments on the Draft *Dioxin Reassessment,* and on EPA's revised versions of Chapter 8 on Dose-Response Modeling as well as the Draft *Integrated Risk Characterization for TCDD*, it is clear that the mechanism of action by which TCDD induces tumors in laboratory animals is unknown or, at best, uncertain. While it is universally accepted that TCDD binds to the Ah receptor, beyond this initial event, the sequence of events leading to tumor development is essentially unknown. In fact, there are no peer reviewed publications which articulate the mechanism of action by which TCDD induces tumors in animals.

TCDD Production and Environmental Concentrations Have Decreased Significantly Over the Last Decade

TCDD has never been intentionally produced other than on a laboratory-scale basis for use in toxicity studies. Rather, it is generated as a by-product of various combustion (some naturally occurring) and chemical processes. Over the last decade TCDD production and environmental concentrations have dramatically decreased due in large part to the discontinued production and use of the herbicide 2,4,5-T, reductions in the manufacture of other chlorinated phenolic compounds, the switch to unleaded automobile fuels, upgraded emission controls for incinerators, and process changes at pulp and paper mills. Additional significant decreases in TCDD production and emissions will result from implementation of recently promulgated emission standards for municipal and medical waste incinerators, and from proposed emission standards for hazardous waste incinerators and for the pulp and paper industry.

EPA has recently conducted a dioxin inventory that assessed dioxin releases to the environment from 1987 to 1995. Although that data has not been published (EPA anticipates releasing this information before the end of the year), it is our understanding that EPA will

demonstrate a greater than 70% decrease in dioxin releases for that time period. EPA's estimate does not include decreased releases since 1995 and the very significant projected emission decreases that will result from compliance with new and proposed regulatory standards. NTP should update its exposure discussion for TCDD to include this new information. NTP may wish to discuss the dioxin inventory data and the projected impact of the new and proposed emission standards with EPA's Office of Research and Development and with relevant EPA Program Offices.

Conclusion

Unlike substances on NTP's current list of *Known Human Carcinogens*, the epidemiological weight-of-the-evidence for TCDD does not adequately fulfill the well recognized causation criteria. Despite a great deal of study on numerous populations exposed to varying amounts of TCDD (some of them having extremely high exposure), the epidemiological data do not permit a conclusion that TCDD is a known human carcinogen. Indeed, three independent reviews (EPA, SAB and IARC) have concluded that the totality of the epidemiological data on TCDD carcinogenicity are only "limited" or "suggestive." Such data do not provide "sufficient evidence" to allow NTP to classify TCDD as a *Known Human Carcinogen.*

The poorly understood mechanism of action for TCDD-induced tumors in laboratory animals, in conjunction with "limited" or "suggestive" epidemiological data, do not support listing TCDD as a known human carcinogen. EPA's Draft *Dioxin Reassessment*, the SAB's extensive review of that document, as well as EPA's revisions to the Draft *Dioxin Reassessment* are all in agreement that beyond Ah receptor binding and enzyme induction, the mechanism by which TCDD induces tumors in animals is poorly understood. At present, there is a significant data gap between Ah receptor binding and enzyme induction and the unknown events which are necessary for the development of tumors. Based on the totality of the available data (epidemiology and mechanism of action), the most that can be concluded is that TCDD can be reasonably anticipated to be a human carcinogen. Clearly, the available data do not support NTP's proposed changing of its current listing of TCDD to the "Known to Be a Human Carcinogen" category.

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